amounts of SDF-1α were produced from the recombinant Sendai Virus and secreted into the culture supernatant. After a 72-hr incubation, the amount of SDF-1α in the culture fluid reached over 10 μg/ml." In addition, Example 4 (pages 11-14), which is entitled "*Assay of biological activities of recombinant chemokines SDF-1α and SDF-1β*," outlines a chemotactic assay, an anti-HIV-1 assay, a cell fusion assay, and a luciferase assay for measuring the biological activity of a chemokine.

REMARKS

The invention features a novel, recombinant Sendai virus vector expressing a pharmaceutically active chemokine in large quantity, e.g., for medical use. Such a recombinant vector can be administered, for example, to human subjects for treatment of HIV infection *in vivo*. Alternatively, the recombinant Sendai virus vectors can be administered *ex vivo* by collecting cells from a human subject, infecting the cells with a recombinant Sendai virus vector expressing the chemokine, and re-introducing the infected cells to the subject.

Claims 1-12, 14, and 15 are pending in the present Continued Prosecution Application. In the parent case, a number of issues were raised by the Examiner. These rejections are addressed by the present amendment and the following remarks.

Rejections under U.S.C. §112, first paragraph

Claim 9 stands rejected under U.S.C. §112, first paragraph, as being broader than the scope of the enabling disclosure of the specification. This rejection is respectfully traversed.

Claim 9 is directed toward a method of treating human immunodeficiency virus infection by administering to human subjects a recombinant Sendai virus vector expressing a biologically active CXC-chemokine and allowing the vector to express the chemokine *in vivo*.

The Examiner states that, because practice of the full scope of the claimed invention would require "undue experimentation," claim 9 is overbroad. Applicants disagree. The legal standard for enablement is articulated in *In re Wands* 858 F 2d. 731, 8 USPQ2d 140, 1402 (Fed. Cir. 1988). *In re Wands* sets forth eight factors to be taken into account to determine whether the experimentation necessary to practice the scope of the claimed invention is "undue." The Examiner has reinterpreted the *Wands* factors to render them more stringent than the statute or the case law, including *Wands*, permits.

The first *Wands* factor is the amount of guidance provided by the specification to practice the invention. The Examiner acknowledges that the specification teaches, as stated on page 9, paragraph 1, that

> "[t]he dose of the virus vector varies depending on the age, weight, and symptoms of the patients, the administration route, and the kinds of chemokines. The virus vector is

> administered at 0.1 to 10,000 virions cell, preferably 0.5 to 50 virions cell."

The Examiner further acknowledges that

> "[t]he specification also provides exemplifications wherein pSeVSDF-1α was transfected to vTR7-3 infected LLCMK2 cells which were subsequently injected into embryonated chicken eggs to amplify the recovered virus, which was then confirmed by SDS-Page, Northern blot, and Western blot, purified via centrifugation. Anti-HIV activity was also demonstrated via incubating MT4 or PHA-stimulated PBMC cells with the purified chemokines then exposing said cells to tissue culture containing HIV-1 (Examples 1&2, pg. 10)."

However, the Examiner reasserts that "the specification fails to disclose [1] the appropriate dose per route of administration, [2] methods of effective targeted delivery of a Sendai virus to any specific cell type, such as CD4 T-cells residing in lymph nodes and brain tissue, and [3] methods of producing a high, stable level of chemokine gene expression." The Examiner further asserts that these elements "are essential for providing *in vivo* gene therapy," and implies that determination of these elements would have beyond the ability of some one in the field to do routinely. Applicants disagree.

The specification teaches a method by which the nondisseminative recombinant Sendai virus vector of the present invention can be used for gene therapy *in vivo*. In order to achieve this, one needs to : (1) identify chemokines having anti-HIV activity; and (2) identify a recombinant viral vector capable of delivering biologically active chemokine to a mammalian host cell. The specification provides ample guidance for these two steps.

The dosage and administration determination mentioned by the Examiner were routine matters that need not be described in detail in the specification.

The specification identifies chemokines having anti-HIV activity. For example, the CXC chemokines, SDF-1α and SDF-1β, are known to inhibit replication of T-cell tropic HIV. In addition, the CC chemokines, MIP-1α and MIP-1β, and RANTES inhibit macrophage tropic HIV strains (page 2, lines 6-9). Moreover, the specification provides abundant, detailed, enabling guidance for producing a therapeutic benefit to a mammal by expressing therapeutic chemokines using the Sendai virus *in vivo*. A summary of such guidance is presented in the table below.

Element of Invention	**Teaching Location in the Specification**
Examples of Sendai virus vectors used in the present invention	page 5, lines 18-21
DNA constructs and construction of recombinant Sendai viral vector	page 9, line 26 to page 10, line 1
Regulation of chemokine gene expression within the Sendai viral vector	page 5, lines 22-31
Modification of the Sendai virus to maintain or improve the vector's expression capability, safety, or efficiency of transcription and replication	page 5, line 32 to page 6 line 18
Generation of nondisseminative Sendai viral vectors	page 6, line 19 to page 7, line 11
Generation of recombinant Sendai viral vectors *in vitro* and *in vivo*	page 7 lines 12-32

Intended cell types	page 7, line 33 to page 8, line 1
Production of chemokines in mammalian cells.	page 10, lines 2-17; page 10, lines 18 to page 11, line 11
Clinical application	page 8, lines 13-22
Pharmaceutical carrier for administration	page 8, lines 23-31
Determination of dose of administration	page 8, line 19; page 9, lines 1-4
Determination of chemokine biological activity (including chemotactic and anti-HIV activity)	page 11, line 29 to page 12, line 16

The Examiner has asserted that a practitioner would require, in addition to the information provided in the specification, demonstration of (1) the appropriate dose per route of administration, (2) methods of effective targeted delivery of a Sendai virus to any specific cell type, such as CD4 T-cells residing in lymph nodes and brain tissue, and (3) methods of producing a high, stable level of chemokine gene expression. Applicants respectfully disagree.

First, as is discussed above, the specification recites viral dosages that can be used with any of the suggested pharmaceutical carriers and administered by any route known in the art. Second, the skilled artisan would be aware of methods that would provide effective, targeted delivery to specific cells, such as cells in the lymph node or brain tissue. The skilled artisan would recognize, for example, that the recombinant Sendai virus of the present invention may be suspended in a saline solution and injected directly into a lymph node of a patient infected with HIV to achieve delivery to CD4 T-cells

residing in the lymph node. Finally, contrary to the Office's assertion that methods of producing a high, stable level of chemokine are not provided, the Applicants have demonstrated that the Sendai virus vector is capable of expressing a high level of a chemokine gene in mammalian cells, that is stable throughout infection and/or through successive passages of the Sendai virus.

In light of the above, the specification provides ample guidance for a person of ordinary skill in the art of gene therapy to carry out an *in vivo* gene therapy procedure according to the present invention. A gene therapy vector is provided by Applicants, as are dosage and a pharmaceutical carrier formulations. Moreover, Applicants have demonstrated that biologically active chemokine can be expressed in mammalian cells (and an embryonated chicken egg) using a gene therapy vector of the invention. Thus, the instructions in the specification provide all of the information needed for the practice of the invention without undue experimentation.

Included in the *Wands* factors is the nature of the invention and the quantity of experimentation necessary to practice the invention.

In re Wands involves a method for identifying monoclonal antibodies that are specific for a particular antigen. The method required screening large numbers of hybridomas to determine which ones secrete an antibody with the desired characteristics. Similarly, each embodiment of the present invention may require testing a variety of predefined parameters in order to optimize the method of *in vivo* gene therapy described

in the specification. All of this sort of optimization is routine in this field. As was stated *In re Wands*, "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed."

The specification provides ample guidance to allow the skilled artisan to optimize a protocol for *in vivo* gene therapy using the recombinant Sendai viral vector. For example, as noted above, the specification teaches a variety of modifications that can be made to the Sendai viral vector to increase the expression of the encoded chemokine (page 5, lines 22-31) or further to "maintain vector's expression capability, inactivate genes for immunogenicity, improve safety, or enhance the efficiency of RNA transcription and replication" (page 5, line 32 to page 6, line 18). In addition, the specification proposes mechanisms by which to alter the disseminative capability of the virus (page 6, line 19 to page 7, line 11). Practitioners in the art routinely test various parameters in order to identify an optimized gene therapy procedure.

Another *Wands* factor is the presence or absence of working examples. As noted above, the Examiner asserts that the specification lacks certain elements that "are essential for providing *in vivo* gene therapy."

It is submitted that the Examiner has interpreted the examples provided in the specification unduly narrowly. Applicants have provided a recombinant Sendai virus and have demonstrated that this virus is capable of producing biologically active chemokine

in mammalian cells which can be further passaged in embryonated chicken embryos. Applicants demonstrated that "[a]fter successive passage in eggs, the recombinant virus reached a titer of over 10^9 PFU/ml, comparable to that of the wild-type Sendai virus" (page 10, lines 9-11). That the high level of expression achieved as described, in Example 1, persisted for successive passages indicates that these levels can be maintained *in vivo*. In addition, Applicants demonstrated that substantial amounts of SDF-1α were produced from the recombinant Sendai virus and secreted into the culture supernatant (10 μg/ml after 72 hour incubation) (see, Example 2). There is no reason to believe that this level of expression would not lead to a therapeutic effect. On the contrary, this example suggests that physiological levels of chemokine can be achieved *in vivo* using the invention. Example 4 demonstrates that the chemokine expressed in mammalian cells by the inventive recombinant Sendai virus is biologically active (i.e., the chemokine displays chemotactic and anti-HIV activity). As discussed in further detail below, the present invention provides the first demonstration, in any cellular expression system, of successful production of a large quantity of chemokine that <u>also</u> maintains biological activity.

Based on the results described above, one of ordinary skill in the art could not fail to recognize that the recombinant Sendai viral vector of the present invention may be used for *in vivo* gene therapy to treat HIV infection in mammals, particularly humans. As is discussed further below, the law does not require that every embodiment of a claim be

operable. Thus, even if a gene therapy procedure exists that would not achieve a therapeutic level of chemokine expression, this would not negate enablement of the present claims.

The next *Wands* factor, level of skill in the art, may be scored in the Applicants favor. The level of skill in the art of gene therapy is high.

Another *Wands* factor is predictability in the art. The Office has asserted that "*in vivo* gene therapy is a highly unpredictable art because there are barriers to the *in vivo* delivery of DNA," which are listed as points (i) through (vii) in the Office Action. Applicants disagree with the basis of this rejection.

Applicants have demonstrated that therapeutically beneficial levels of biologically active chemokine proteins can be produced in mammalian cells in culture and in a living mammalian embryo. It is entirely predictable that such *in vivo* protein production will provide therapeutic benefit. Noting general failures, of others, not the inventors, in the field of gene therapy does not explain why the proven, *in vivo* effects of the invention should be held suspect.

Under the standard of enablement, an Applicant is required to provide sufficient information to allow practice of the invention, not to prove that all possible embodiments of the invention will work.

Finally, the Examiner applies a standard of perfection with respect to enablement that finds no basis in the statute or the case law. The Office requires that every

conceivable embodiment falling within the claims perform successfully, with failures in thought experiments negating enablement. If this were the standard for enablement, generic claims would never be allowable, in any instance in which an Examiner can imagine a single inoperative embodiment. This is not the standard the law requires. For example, in *Application of Angstadt*, 537 F.2d 498, 190 U.S.P.Q. 218 (C.C.P.A 1796), the Court, in holding that a claimed invention was enabled, even though the claims admittedly included inoperative embodiments, stated that "the evidence as a whole, including the inoperative as well as operative example, negates the PTO position that persons of ordinary skill in this art, given its unpredictability, must engage in undue experimentation to determine which complexes work."

Rejections under 35 U.S.C. §103(a)

Claims 1-8 and 11-15 stand rejected under 35 U.S.C. §103(a) for obviousness over Hasan et al. (1997) or Yu et al. (1997) in view of Bleul et al. (1996) and Calain et al. (1993). This rejection should be withdrawn.

Prior to the present invention, as disclosed in the specification (page 2, lines 19-21), purification of recombinant, biologically active chemokines expressed in mammalian and other higher vertebrate cells by recombinant viruses had not been reported, by anyone in the field. Typically, recombinant highly basic polypeptides, including chemokines, were expressed in *E. coli* with low productivity in processes that required extensive, multi-step purification of the product before use. Furthermore, in these systems,

extensive aggregation was often inevitable, particularly for such basic polypeptides as chemokines. Such aggregation resulted in production of inactive chemokine polypeptides.

The present invention is based on the discovery that a recombinant Sendai virus vector can be engineered to express large quantities of non-aggregated, biologically active chemokine in mammalian host cells. The maintenance of biological activity is a key feature of the invention.

None of Hasan et al., Yu et al., Bleul et al., or Calain et al., alone or in combination, disclose or suggest expression of large quantities of non-aggregated, biologically active chemokine in mammalian cells using the Sendai virus.

Hasan et al. describes the expression of the firefly luciferase gene using a recombinant, non-segmented, negative strand RNA Sendai virus. Hasan et al. expressly state that the expressed firefly luciferase protein was not biologically active. For example, on page 2818, column 2, lines 9-20, the authors state:

> "The luciferase gene product (62 kDa) became detectable at 6 h p. i., and then increased in amount (Fig. 3B). Its production kinetics appeared to correlated well with that of luciferase activity in cells as shown in Fig. 2(A). Later in infection, this band did not increase in intensity. Instead, high molecular mass material appeared between the stacking, and separating gels, and the amount increased with time (data not shown). This material most likely represented the intracellular aggregation of expressed and accumulated luciferase molecules. These aggregates could be enzymatically inactive and their preferential accumulation would account for the fact

that luciferase activity did not increase beyond 14 h p.I. (Fig. 2)."

Later, the authors conclude,

> "we have been unable to assess the maximum capability of SeV to express a foreign functional protein by this particular reporter gene because of inactivation of the gene products, probably due to extensive aggregation in the cells."(emphasis added)

Based on the teachings of Hasan et al., if considered as a whole, one skilled in the art would not be motivated to choose Sendai viral vectors to express chemokines, which, as noted above, are known to be susceptible to inactivation due to intracellular aggregation in *E. coli*. Hasan et al. do not disclose or suggest the recombinant Sendai virus necessary to practice the invention of claims 1-8 and 11-15.

Yu et al. teach the use of the Sendai virus-based expression system of Hasan et al. to produce high levels of gp120 in CV1 cells (2.0 μg/ 10^6 infected cells using the standard V(+) version and 6.0 μg/10^6 infected cells using the V(-) version of the Sendai virus). Yu et al. do not disclose or suggest that a Sendai virus system may be used to express large quantities of a chemokine that maintains its biological activity, as recited in claims 1-8 and 11-15. In contrast, Yu et al. teach away from this concept by referring to Hasan et al. (who failed to obtain biologically active system), and stating that "no definite assessment of the utility of the SeV vector has been made because of inactivation of the enzyme

activity due to extensive aggregation of the expressed luciferase molecules in cells" (page 463, column 2, lines 25-29). Thus, one skilled in the art would not be motivated to express a chemokine, known to be susceptible to intracellular aggregation, using a Sendai virus, based on the Yu et al. reference.

In addition, Hasan et al. refer to Yu et al. and states that "the SeV/gp120 recombinant displayed a very similar attenuated phenotype to that of SeV/luc" Hasan et al. page 2819, column 1, lines 30-31.

The secondary reference Bleul et al. demonstrates the anti-HIV activity of SDF-1α. Chemotaxis assays described in Bleul et al. utilize synthetic human SDF-1α folded by air oxidation and purified using reverse phase HPLC. Bleu et al. also refers to a murine SDF-1α purified from a murine bone-marrow stromal cell line that is used as a standard control. Both of these methods typically produce small quantities of protein whose use is limited to experimental purposes.

The specification of the present application teaches, generally, that chemical synthesis of basic polypeptides, such as chemokines, is typically used because extensive aggregation during cellular expression is often inevitable (page 2, lines 19-30). In addition, the present application points out that this approach is not only laborious, requiring careful refolding, but also expensive (page 2, lines 19-30).

Bleul et al. does not disclose or suggest use of a Sendai viral vector, as presently recited in claims 1-8 and 11-15, to produce substantial amounts of a biologically active

chemokine in mammalian cells. In fact, since the chemokines used in the experiments described in Bleul et al. are obtained by tedious and expensive methods that yield small quantities of protein, the teachings of Bleul et al. could reasonably have led the skilled artisan to conclude that no recombinant methods were available prior to the present invention to produce substantial amounts of biologically active chemokines in mammalian cells. Certainly, Bleul et al. does not provide the requisite information necessary to generate the high levels of biologically active chemokines using the claimed Sendai virus vectors.

Finally, Calain et al. as noted by the Examiner, describes recovery of Sendai virus nucleocapsids using centrifugation. Calain et al., like Bleul et al., do not provide the requisite information, when combined with the primary references, necessary to generate the high levels of biologically active chemokines by the claimed Sendai virus vectors.

In contrast to the prior art, Applicants have discovered that a Sendai virus vector can be used successfully to express substantial amounts of a biologically active chemokine in mammalian cells. This ability to produce biologically active chemokine represents a significant advance, particularly in fields such as the field of HIV treatment, where the delivery of anti-HIV chemokines has been limited by the inability to express substantial amounts of biologically active chemokines in mammalian cells. Nowhere do Hasan et al., Yu et al., Bleul et al., or Calain et al. disclose, suggest, or provide either the guidance or the motivation for creating such a Sendai virus vector. Indeed, the

combination of these references would actually discourage the skilled artisan from using a Sendai virus vector to express an aggregation-prone chemokine.

Because none of Hasan et al., Yu et al., Bleul et al., or Calain et al. disclose or suggest the Sendai viral vectors of the presently claimed invention that are necessary for production of substantial amounts of biologically active chemokines, these references cannot be properly combined to support an obviousness rejection of claims 1-8 and 11-15. Reconsideration of claims 1-8 and 11-15 and withdrawal of this rejection is respectfully requested.

Claim 10 stands rejected under 35 U.S.C. §103(a) for obviousness over Hasan et al. (1997) in view of Bleul et al. (1996) and Hasegawa (1997).

Claim 10 recites a method of treating human immunodeficiency virus infection by collecting target cells from human subjects, infecting the cells with a recombinant Sendai virus vector expressing substantial amounts of a biologically active CXC-chemokine, and administering the infected cells to the human subjects.

As stated above, Hasan et al. teach expression of the firefly luciferase gene using a recombinant, non-segmented, negative strand RNA Sendai virus. The Hasan et al. reference states that the firefly luciferase protein produced lacks biological activity. Based on these teachings, one skilled in the art would not be motivated to choose a Sendai viral vector to express an aggregation-prone chemokine for use in an *ex vivo* gene therapy method of treating human immunodeficiency virus infection. This reference

alone or combined with the other cited references, clearly does not disclose or suggest use of a Sendai virus vector to practice the method of claim 10.

As described above, although the secondary reference, Bleul et al., demonstrates the anti-HIV activity of SDF-1α, Bleul et al. does not disclose or suggest use of a recombinant Sendai virus to express substantial amounts of a biologically active chemokine as recited in claim 10. Bleul et al. merely describes chemotaxis assays that utilize small quantities of synthetic human SDF-1α or native, endogenous SDF-1α purified from a murine bone-marrow stromal cell line. Thus, Bleul et al. do not provide the information necessary to generate the recombinant Sendai viruses that are capable of producing high levels of biologically active chemokine.

Hasegawa et al. teach use of the Sendai virus in gene therapy. Hasegawa et al. do not suggest that the Sendai virus can be used as a gene therapy vector to provide proteins, such as chemokines, that are susceptible to inactivation by aggregation. Hasegawa et al. do not disclose or suggest that a biologically active chemokine can be produced at sufficient levels to deliver a therapeutic benefit to a patient infected with HIV.

Thus, none of Hasan et al., Bleul et al., or Hasegawa et al. disclose or suggest the use of the Sendai virus to express therapeutically beneficial quantities of biologically active chemokine in a method of *ex vivo* gene therapy. Indeed, the combination of these references teaches away from using the Sendai viral vector as an *ex vivo* gene therapy vector for expression of biologically active highly basic chemokines, which the prior art

teaches are highly basic proteins that are likely to aggregate.

Conclusion

In view of the above, applicants submit that the claims are now in condition for allowance, and such action is requested. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: June 6, 2002 for Mary Rose Scozzafava Reg No. 36,268

Paul T. Clark
Reg. No. 30,162

Clark & Elbing LLP
176 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045

Ntserver documents 50026 50026.008001 Preliminary Amendment CPA.wpd

Version with Markings to Show Changes Made

Amend claims 10 and 15 as follows.

10. (Twice Amended) A method of treating human immunodeficiency virus infection, which comprises collecting target cells from human subjects, infecting the cells with a recombinant Sendai virus vector expressing a substantial amount of biologically active CXC-chemokine, and [giving] returning the infected cells [back] to the human subjects.

15. (Twice Amended) A method of inhibiting proliferation of HIV-infected cells *in vitro* [proliferation] which comprises, incubating the host cell of claim 14 [in vitro] *in vitro* under conditions that [to] allow for secretion of a substantial amount of biologically active chemokine; and contacting said chemokine with cells that are infected with HIV, thereby inhibiting proliferation of HIV-infected cells *in vitro*.

Clean Version of all Pending Claims

1. (Amended) A recombinant Sendai virus vector expressing a substantial amount of biologically active chemokine.

2. The recombinant Sendai virus vector of claim 1, wherein said chemokine is CXC-chemokine.

3. The recombinant Sendai virus vector of claim 2, wherein said CXC-chemokine is stromal cell-derived factor α or stromal cell-derived factor β.

4. The recombinant Sendai virus vector of claim 3, wherein said vector is disseminative.

5. The recombinant Sendai virus vector of claim 3, wherein said vector is infectious and replicates autonomously, but is not disseminative.

6. (Amended) A method of producing a substantial amount of biologically active chemokine which comprises inserting at least one chemokine

gene into a Sendai virus vector, allowing the vector to produce said chemokine, and recovering said chemokine.

7. The method of claim 6, wherein said chemokine is CXC-chemokine.

8. The method of claim 6, wherein the step of recovering comprises the step of removing virions by centrifugation.

10. (Twice Amended) A method of treating human immunodeficiency virus infection, which comprises collecting target cells from human subjects, infecting the cells with a recombinant Sendai virus vector expressing a substantial amount of biologically active CXC-chemokine, and returning the infected cells to the human subjects.

11. (Amended) A pharmaceutical composition comprising a recombinant Sendai virus vector expressing a substantial amount of biologically active stromal cell-derived factor α or stromal cell-derived factor β and a pharmaceutically acceptable carrier, wherein said vector is disseminative.

12. (Amended) A pharmaceutical composition comprising a recombinant Sendai virus vector expressing a substantial amount of biologically active stromal cell-derived factor α or stromal cell-derived factor β and a

pharmaceutically acceptable carrier, wherein said vector is infectious and replicates autonomously, but is not disseminative.

14. (Amended) A host cell transfected with a recombinant Sendai virus vector expressing a substantial amount of biologically active chemokine.

15. (Twice Amended) A method of inhibiting proliferation of HIV–infected cells *in vitro* which comprises, incubating the host cell of claim 14 *in vitro* under conditions that allow for secretion of a substantial amount of biologically active chemokine; and contacting said chemokine with cells that are infected with HIV, thereby inhibiting proliferation of HIV-infected cells *in vitro*.